frontiers

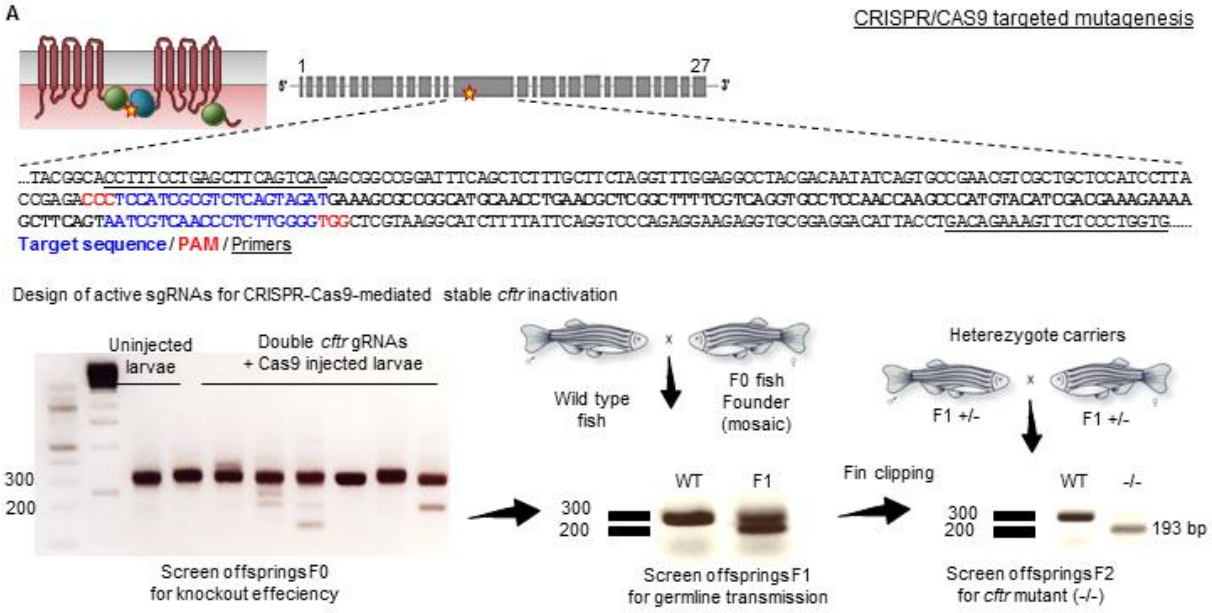
Supplementary Material

Supplementary Figures

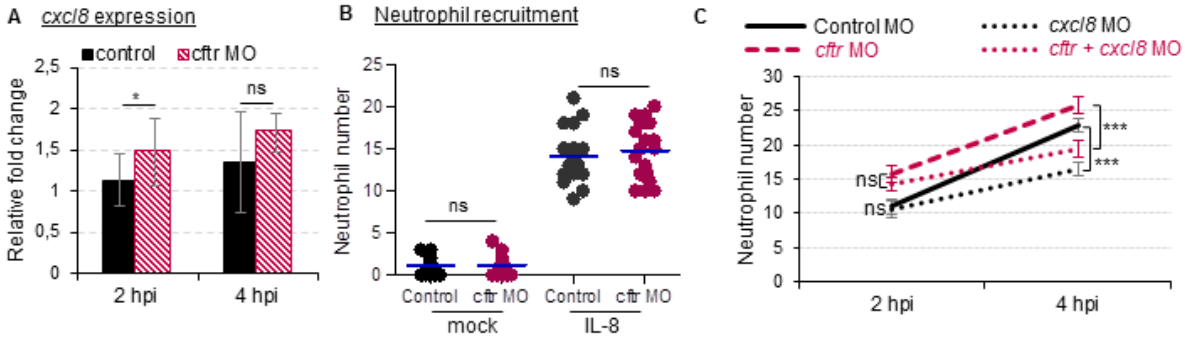
Supplementary Figure 1. Generation of CFTR-depleted zebrafish
(A) Generation of *cftr* null mutant in zebrafish using CRISPR-Cas9 gene editing. Schematic of the domain structure of CFTR and with a star indicating the CRISPR-Cas9 target site. Double guide RNA (gRNA) for CRISPR-Cas9-mediated stable *cftr* inactivation have been designed to effectively induce a deletion in *cftr* gene. The expected targeted deletion (127 bp) in F0 founder fish and germline transmission were detected and confirmed by PCR Analysis. Heterozygous carriers for the mutation are crossed to obtain *cftr* $^{-/-}$ homozygous mutant (#sh540). *cftr* -/- mutants are screening for impaired Kupffer's vesicle inflation at 8-somite stage. (B) Loss of CFTR causes Kupffer's vesicle developmental defects in zebrafish. Representative photomicrography showing altered Kupffer's vesicle inflation in both *cftr* -/- mutant and *cftr* morphant.

Supplementary Figure 2. IL8-dependent neutrophil chemotaxis in *cftr*-defective zebrafish larvae
(A) Tail transection was performed on control and *cftr* MO and mRNA levels of *cxcl8* gene determined by qRT-PCR in tail fin tissue at 2 and 4 hpi. Gene expression was normalized against *ef1a* and expressed as fold change over tail fin tissue from uninjured larvae (30 fins per replicate; mean relative ± SEM gene expression of 4 independent experiments; two-tailed Bonferroni t-test). (B) Mean number of recruited neutrophils into the otic cavity in response to mock or IL8 injection in control and *cftr* MO *TgBAC(mpx:EGFP)i114 larvae* monitored at 2 hpi (n=20; means ± SEM from 3 independent experiments; two-tailed Bonferroni t-test). Cell counts in control or CFTR-depleted animal show no significant difference (ns) in the average number of neutrophils recruited at IL8-injected site. (C) *TgBAC(mpx:EGFP)i114* controls, *cftr, Il8,* and double *cftr/il8* morphants were tail amputated and neutrophils at wounds were enumerated at 2 and 4 hpi (n=21; means ± SEM from 3 independent experiments; two-way ANOVA with Tukey post-test).

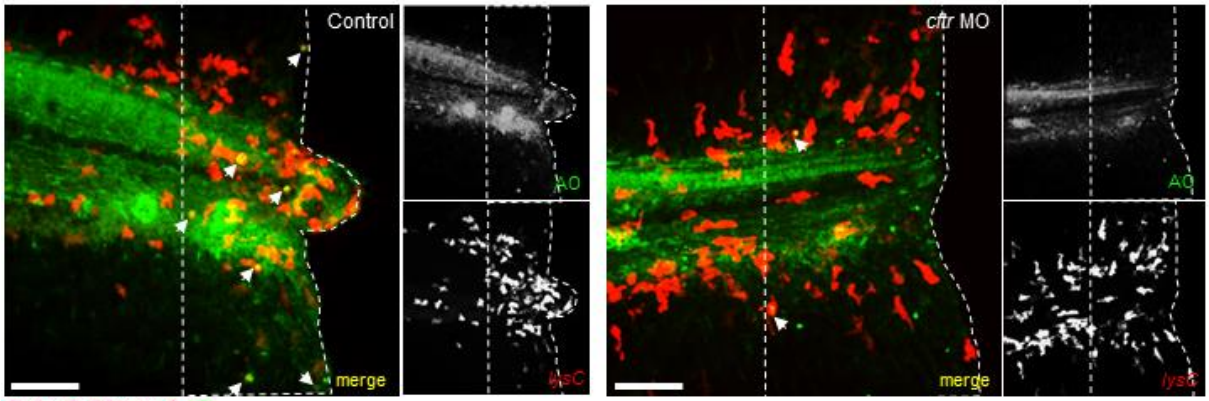
Supplementary Figure 3. Neutrophil lifespan in *cftr*-defective zebrafish larvae
3 dpf control and *cftr* MO *Tg(LysC:DSred)nz5* larvae were amputated and stained with acridine orange (OA) to label death cells. Representative confocal pictures of injured tails at 8 hpi (scale bars, 60 µm) revealing the proportion of dying neutrophils at the wound (white arrow). Dotted lines indicate the outline of the wounds.


A
CRISPR/CAS9 targeted mutagenesis
1
27
5'
3'
...TACGGCACCTTTCCTGAGCTTCAGTCAGAGCGGCCGGATTTCAGCTCTTTGCTTCTAGGTTTGGAGGCCTACGACAATATCAGTGCCGAACGTCGCTGCTCCATCCTTA
CCGAGACCCTCCATCGCGTCTCAGTAGATGAAAGCGCCGGCATGCAACCTGAACGCTCGGCTTTTCGTCAGGTGCCTCCAACCAAGCCCATGTACATCGACGAAAGAAAA
GCTTCAGTAATCGTCAACCCTCTTGGGGTGGCTCGTAAGGCATCTTTTATTCAGGTCCCAGAGGAAGAGGTGCGGAGGACATTACCTGACAGAAAGTTCTCCCTGGTG.....
Target sequence / PAM / Primers
Design of active sgRNAs for CRISPR-Cas9-mediated stable cftr inactivation
Uninjected larvae
Double cftr gRNAs + Cas9 injected larvae
300
200
Screen offsprings F0 for knockout effeciency
Wild type fish
F0 fish Founder (mosaic)
WT
F1
300
200
Screen offsprings F1 for germline transmission
Fin clipping
Heterezygote carriers
F1 +/-
F1 +/-
WT
-/-
300
200
193 bp
Screen offsprings F2 for cftr mutant (-/-)
B
Kupffer's vesicle
12 hpf
WT
cftr -/-
cftr MO

S1


A
cxcl8 expression
control
cftr MO
Relative fold change
2,5
2
1,5
1
0,5
0
*
ns
2 hpi
4 hpi
B
Neutrophil recruitment
Neutrophil number
25
20
15
10
5
0
ns
ns
Control
cftr MO
Control
cftr MO
mock
IL-8
C
Control MO
cftr MO
cxcl8 MO
cftr + cxcl8 MO
Neutrophil number
30
25
20
15
10
5
0
ns
ns
***
***
2 hpi
4 hpi

S2



Tg(LysC_DSred)nz5 AO

S3